## REMARKS

Claims 1, 3, 7-11, 17-32, and 33-38 were pending in the present application. Claims 17-32 had been withdrawn pursuant to a restriction requirement. Claims 37-38 were withdrawn as directed to a non-elected invention. Claims 17-32 and 37-38 have been canceled herein. Claims 39 and 40 have been added. Upon entry of the present amendment, claims 1, 3, 7-11, 33-36, and claims 39-40 will be pending.

All pending claims were rejected in the Final Rejection. Applicant submitted an amendment on June 25, 2007. In the Advisory Action dated as mailed July 9, 2007, the Office advised that the amendments would not be entered for purposes of appeal, but also noted that the rejection for nonstatutory obviousness-type double patenting had been withdrawn in view of the terminal disclaimer filed on June 25, 2007. This paper responds to the Final Rejection and Advisory Action.

Preliminarily, Applicant notes with the appreciation the withdrawal of the objections to the drawings and the specification in the Final Rejection. Applicant also notes with appreciation the withdrawal of the rejections over Longberg et al. (U.S. Pat No. 5,874,299) and Riechmann et al.; over Longberg et al. and Riechmann et al., further in view of Longberg II (U.S. Pat. No. 5,625,126) and over Longberg et al. and Riechmann et al., further in view of Green et al.

Also, amendments have been made consistent with amendments made in corresponding Application Serial No. 10/693,308, following a telephonic interview in that application ("the '308 Interview") discussing many of the same issues present in this application. Specifically, the claims have been amended to recite the method steps in an explicit manner, and to include a recitation specifying how the expression of heterologous VHH heavy chain locus specifically in

B cells will be achieved, i.e., through the incorporation of a regulatory sequence providing for expression of the VHH heavy chain locus specifically in B cells. Support for the positive method steps is implicit from the claims as originally filed; support for the recitation regarding B cell expression can be found, *inter alia*, on page 22, line 19 through page 23, line 14, of the application as filed.

For purposes of clarification, the word "region" has been changed to "exon" in claims 1 and 3, and claims 7 and 8. Both terms are used interchangeably in the specification, but the latter term is more accurate. (See, for example, page 9, lines 24-29 -- "A 'VHH exon/region' in the context of the present invention describes a naturally occurring VHH coding sequence . . ." )

It is Applicant's belief that the foregoing amendments also address the rejection for lack of enablement, in conjunction with the arguments advanced during the '308 Interview, as discussed herein. It became apparent during the '308 Interview that the Office was of the view that the Janssens et al. reference, cited in the previous response to support enablement, was limited to the situation in which an IgM knock out mouse is used, and the Office further asserted that such animals are not disclosed in the specification. Accordingly, the Office took the position that the claims were not enabled. As was pointed out during that interview, however, the Janssens et al. reference is not limited to the situation in which an IgM knock out mouse is used. Specifically, see the sentence bridging columns 1-2 on page 15132, emphasis added, – "Moreover B220/CD19-positive BM cells of GΔ line 1 transgenic mice in a WT background . . ."

While recognizing that Janssens et al. describes expression in wild type mice in the Advisory Action in the 10/693,308 application, the Office nonetheless took the position that

Janssens et al. only shows the expression of GΔ in wild type mice, not MΔGΔ, and only shows immunization in mutant mice. The Office seems to be of the view that using wild type mammals will negatively impact immunization. This view is incorrect.

As set forth in the Declaration of Louis M. Weiner, M.D. ("Weiner Declaration"), paragraph 16, the Office provides no scientific reason to conclude that the MΔGΔ construct would not also be expressed in wild type. Indeed, the data in the Declaration of Dr. Grosveld ("Grosveld Declaration"), cited in the Weiner Declaration, proves otherwise. The data show the expression of the MΔGΔ locus in a wild type background (Grosveld Declaration, paragraph 5, Figure 2). As also set forth in the Weiner Declaration, the Office provides no scientific reason to conclude that the production of antibody in response to antigen challenge can only occur in mutant mice that do not produce light chain-containing antibodies – indeed camelids produce both single heavy chain only antibodies and antibodies that include light chains (Weiner Declaration, ¶¶ 16 and 17.)

Regardless, there is ample disclosure in the present specification regarding the use of knock out mice. See, for example, page 8, lines 10-14, wherein the deletion or silencing of the heavy chain loci endogenous to the mammal is disclosed, citing various references; page 28, lines 23-28, wherein it is stated that it is preferred that the recipient animal may be a "knock out" animal that is capable of having one or more of the genes required for the production of antibodies with light chains turned off or suppressed; and page 24, lines 2-3, wherein the inactivation of the endogenous locus is disclosed. Although these recitations do not specify that the animal be an IgM knock out, IgM knock out mice are specifically disclosed in Figure 1 of the application as filed.

In addition to the foregoing amendments, two new claims are submitted for consideration – claims 39 and 40. In view of the foregoing cancellation of claims, the net number of claims has not increased. Support for new claim 39 can be found, *inter alia*, on page 8, line 6. Support for new claim 40 can be found, *inter alia*, on page 23, lines 12-13.

Other amendments have been made for clarity, to change dependencies, or to include recitations from other claims.

The foregoing amendments were not made previously as the relevance of some did not become apparent until the Final Rejection, and the relevance of others did not become apparent until a telephonic interview with the Office in Application Serial No. 10/693,308 discussed below.

Although Applicant believes the foregoing amendments should overcome the outstanding rejections, the rejections will be addressed below.

**Rejection Under 35 U.S.C. § 112, first paragraph**

Claims 1, 3, 7-8, 10-11, and 33-36 were rejected under 35 U.S.C. § 112, first paragraph, as allegedly failing to comply with the enablement requirement. Applicant traverses this rejection.

As an initial matter, the Office again stated that "the specification provides **only prophetic** and a general methodology without disclosing any specifics." (*See* Final Rejection, page 5, emphasis added.) This statement is not only inaccurate, but it also misstates the requirement for enablement. First, the specification does provide specifics, to the degree needed to practice the invention. See the discussion of Janssens et al. below. Second, working examples are not required.

> Compliance with the enablement requirement of 35 U.S.C. 112, first paragraph, does not turn on whether an example is disclosed. An example may be "working" **or** "prophetic." A working example is based on work actually performed. A prophetic example describes an embodiment of the invention based on predicted results rather than work actually conducted or results actually achieved.

MPEP § 2164.02, emphasis added.

Once again, the Office asserted three aspects it considered broad: the breadth of subject population for expressing VHH heavy chain locus; any vector comprising any promoter or locus control region for expressing VHH specifically in B cells; and using ES cells of any species and expressing VHH locus. Regarding the first aspect, the Office relied upon De Genst et al. to support its position. The Office asserted that the teaching of De Genst et al. suggest that the mechanism of pre B cell maturation for the production of VHH single heavy chain was not known, and that it was not apparent from the specification whether a method as recited in the claims would result in a fully functional IgG molecule. As discussed during the '308 Interview, however, De Genst et al. has been countered by Janssens et al. As set forth more clearly below, Janssens et al. followed the procedures set out in the specification. And, as noted by Dr. Weiner, Janssens et al. shows functional expression of several mu and gamma single heavy chain isotypes, all of which bind antigen (Weiner Declaration, ¶18).

Additionally, as discussed during the '308 Interview, at the time of filing, it was known that certain regulatory elements, i.e., LCRs, function across species. See, U.S. Patent No. 5,770,398. Applicant also includes herein the Declaration of Rudolph Grosschedl, Ph.D. ("Grosschedl Declaration").

There is no scientific evidence to suggest that natural IgH regulatory

> elements active in one non-human mammal will not be active in another. In fact, the IgH regulatory elements have been well characterized, and I am not aware of reports in which a gene that is expressed faithfully in one mammal is not expressed in another mammal. Rare cases, in which poor expression has been reported, do not reflect the field and are typically due to the use of incomplete constructs.

(Grosschedl Declaration, ¶ 15.) Indeed, human heavy and light chain immunoglobulin gene loci have been expressed as transgenes in both mice and cattle (Grosschedl Declaration, ¶ 11). The mouse, thus, provides a valid model to evaluate gene expression with the expectation that if a gene is functionally expressed in the mouse it will also be expressed as a transgene in other non-human mammals (*Id.*). Applicant respectfully submits that, at the time of filing, in light of the specification, and that which was well known to those of ordinary skill in the art (Grosschedl Declaration, ¶ 17), one of ordinary skill in the art could have practiced the claimed method in any non-human mammal, without undue experimentation.

Regarding the second aspect, the Office relied upon Keefer and Hammer et al., focusing upon the differences in levels of expression and the resultant phenotype depending upon the promoter used. As also discussed during the '308 Interview, however, these references are not relevant to the present invention. Applicant is not seeking a similar phenotype; all that is needed is sufficient antibody production for isolation. Applicant achieves this.

> All that is required is sufficient gene expression to isolate the antibody mRNA or antibody . . . Dr. Grosveld's declaration shows that human heavy chain-only antibody mRNA can be isolated and sequenced from the B-cells present in a few microlitres of blood, and this against a background of B-cells producing endogenous mouse antibody.

(Weiner Declaration, ¶ 20.) The technology to do so was routine at the priority date (Weiner Declaration, ¶ 21).

Regarding the third aspect, as Applicant stressed during the '308 Interview, it is not necessary to use ES cells. As reported in Janssens et al., fertilized eggs can be used. The non-necessity of ES cells is further confirmed by Dr. Grosschedl:

> I would expect that **any** route of introduction of the vector into the germ-line of **any** mammal would result in heavy chain antibody production by B-cells.

(Grosschedl Declaration, ¶ 16, emphasis added). Thus, the argument that ES technology is limited to mice is irrelevant.

During the '308 Interview, it became apparent that the Office was not convinced that Applicant disclosed the same process used in Janssens et al. to prepare the animals in his specification. To assist the Office in its assessment, Applicant is duplicating the portion of the paragraph regarding Janssens et al. submitted with the previous response addressing preparation of the animals, but including citations to Applicant's application as filed. Specifically, the cited paper discloses the expression of loci containing IgM and IgG, and IgG only (page 7, line 21, through page 8, line 3), human constant regions (Figure 1 and page 11, lines 1-11) , lacking CH1 (page 11, lines 9-11 and page 24, lines 17-20), with two camelid VHH regions, and human D and J regions (page 12, lines 13-19) in mice (page 28, line 3). Bac clone 11771 and pFastBac were both used successfully (page 22, lines 4-5). The loci further contained FRT and LoxP sites (page 21, lines 16-19), and immunoglobulin LCR (page 23, lines 12-14). The vectors were injected into fertilized mouse eggs of animals (page 27, lines 24-30) that do not produce surface IgM and have a block in B cell development at the pre-B cell stage (*see* page 28, lines 23-28 and Figure 1), and in wild type mice (page 27, lines 21-23).

Another one of the Office's repeated concerns has been "the random nature of transgene

insertion" (*see* page 12 of the Final Rejection) and its effect on expression. As discussed during the interview, however, one can achieve position independent expression through the use of, for example, LCRs. See, U.S. Patent No. 5,770,398. LCRs are not, however, required. *See* Grosschedl Declaration, ¶ 12. Without LCRs, the technology will still work, expression is less efficient and more variable, and more animals may need to be screened. Applicant submits that such is not undue experimentation but, rather, routine for one of ordinary skill in the art.

> The test is not merely quantitative, since a considerable amount of experimentation is permissible, if it is merely routine, or if the specification in question provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed.

MPEP § 2164.06, citing *In re Wands*, 858 F.2d 731, 737, 8 USPQ2d 1400, 1404 (Fed. Cir. 1988) (citing *In re Angstadt*, 537 F.2d 489, 502-04, 190 USPQ 214, 217-19 (CCPA 1976)).

Another concern that came out from the '308 Interview, as set forth in the Interview Summary dated as mailed June 1, 2007 ("the '308 Interview Summary"), is the effect of copy number on transgene expression. But, such had already been addressed by the art at the time of filing of the present application. See, for example, the discussion on page 21, lines 20-23, of the application as filed, which discusses the use of FRT sites to allow production of single copy transgenics.

Although using micro-injection in fertilised eggs to generate transgenic animals does not allow precise control of the number of copies of the transgenic locus that will be inserted, it was well known in the art as of the priority date of this application that injection of low concentrations of the DNA into the fertilized eggs favors single copy or low copy number integrations. See, for example, Strouboulis et al., *Genes and Development*, 6:1857-1864, 1992,

page 1861, column 2, last sentence of first paragraph (copy enclosed).

Further, if LCRs are used, each copy contains an LCR and would be expressed at normal levels. In the case of most genes, this could lead to an overexpression of the gene(s) in the transgenic animal. For the Office's convenience, Applicant has included a copy of a review of LCRs, published in October, 1999 (i.e., well before Applicant's priority date) – Li et al., "Locus control regions coming of age at a decade plus," *TIG*, 15(10);403-408, 1999. As is clear from this review, expression with LCRs is copy number dependent, i.e., the greater the copy number, the greater the expression (see page 404, column 2).[1] Applicant notes, however, that in the case of the immunoglobulin loci, the copy number is not particularly relevant because of the nature of the immune response. Once a productive VDJ recombination takes place the recombination process is stopped and only one of the loci is expressed. The evidence that copy number is indeed not relevant in the heavy chain only antibody transgenic mice was obtained by building in recombination sites that allowed the conversion of a five copy animal into a single copy animal. Heavy chain only antibodies are produced equally effectively in these animals, which carry the multicopy or single copy locus at the same insertion site (*see* Janssens et al., 2006, column 1, page 15131, through column 1, page 15132, section entitled "Chimeric Loci Lacking a Human CH1 region," as well as the section referenced above).

Applicant respectfully requests that this rejection be withdrawn.

**Rejection Under 35 USC 112, second paragraph**

[1]Applicant has again included a form PTO/SB/08b listing both the Li et al. and Strouboulis et al. references and again requests that the Office mark the same as being considered. The fee was paid previously and is not being resubmitted. Applicant has not included additional copies of the references as it is believed that they were already received. If Applicant's belief is erroneous, the Office is asked to inform the undersigned and copies will be resubmitted.

Claims 1, 3, 7-8, 10-11, and 33-36 were rejected under 35 USC 112, second paragraph as allegedly being incomplete for omitting essential steps. Specifically, the Office noted that the steps of how expression of heterologous VHH heavy chain locus specifically in B cells of the nonhuman mammal would be achieved were missing. Claims 1 and 3 have been amended to recite that the locus contains a regulatory sequence providing for expression of the VHH heavy chain locus specifically in B cells, and to positively recite the method steps of immunizing the animal and isolating antibody. The former amendment was submitted with a proposed amendment in Application Serial No. 10/693,308. The '308 Interview Summary indicated that the recitation submitted with the proposed amendment would overcome this same rejection in that application.

Applicant respectfully requests that this rejection be withdrawn.

**Rejection Under 35 USC 102**

Claims 1-2 were rejected under 35 USC 102(b) as allegedly anticipated by Ledbetter et al (WO 99/42077). Claim 1 has been canceled. Claim 2 recites that the locus contains a regulatory sequence providing for expression of the VHH heavy chain locus specifically in B cells and that "the VHH exon, the D exon and the J exon are **capable of recombining** to form VDJ coding sequence." Neither limitation is disclosed or suggested by Ledbetter et al. Ledbetter et al. describes the isolation of an already recombined VHH locus. Indeed, in the '308 Interview Summary, the Office acknowledged that the first amendment would overcome this same rejection in that application.

Regardless, Ledbetter et al. does not disclose a method to produce VHH antibodies in a transgenic animal in response to antigen challenge (Weiner Declaration, ¶¶ 22-25). Although

Ledbetter et al. proposes that transgenic mice expressing VH binding domains may be used to produce VHH to any antigen by immunization, this is technically impossible as the VHH binding domains are transcribed from already rearranged llama genes (Weiner Declaration, ¶ 23). Such a rearranged gene can be expressed in a mouse background, but it retains all the features of the antibody originating from the camel (Weiner Declaration, ¶ 24, citing Zou et al.).

Applicant respectfully requests that this rejection be withdrawn.

**Rejection for Obviousness-type Double Patenting**

Claims 1, 3, 7-8, 10-11, and 33-36 were provisionally rejected for obviousness-type double patenting in view of claims 1-4, 7-11, and 33-36, of copending Application No. 10/693,308. A terminal disclaimer was filed on June 25, 2007. It is Applicant's understanding that this rejection has been withdrawn in view thereof.

The Office listed four references as not relied upon but considered pertinent to Applicant's disclosure. Two references have already been cited in rejections which have been withdrawn. Applicant reserves the right to comment on these references if, and when, they are relied upon by the Office.

## CONCLUSION

Applicant respectfully submits that claims 1, 3, 7-8, 10-11, 33-36, and 39-40 are in condition for allowance. An early notice of the same is earnestly solicited. The Examiner is invited to contact Applicants' undersigned representative at (215) 665-5593, if there are any questions regarding Applicants' claimed invention.

Respectfully submitted,

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